Investigation of severe analytical interference in an enzymatic creatinine assay in a case of paracetamol overdose

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Introduction

Paracetamol is metabolised into glucuronide and sulphate conjugates, until saturation at higher doses, where it is oxidised to the toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI).

NAPQI is a hepatotoxic electrophilic molecule which binds thiol groups on proteins, forming adducts, inducing hepatocellular damage.

Most laboratories use enzymatic creatinine assays (ECre). Literature and manufacturer documents suggest negative interference by paracetamol, NAPQI, and the treatment N-acetylcysteine (NAC) in these assays. Which may lead to missed renal injury in paracetamol overdose.

Case Study

An 88-year-old female with an unremarkable past medical history attended the Emergency Department. She was discovered unconscious (GCS 3/15) with empty packets of paracetamol (64 g) and dihydrocodeine (480 mg) and it was thought the overdose was taken 6 h before admission.

IV fluids and NAC were administered and she was admitted to ITU. She was not a candidate for liver transplant due to rising bilirubin. Advice was sought from a specialist liver centre, who suggested haemofiltration with NAC.

Initial biochemistry showed an unmeasurable concentration of serum creatinine (Table 1); this arose suspicion as previous was 53 µmol/L, one month previous.

Time Since Ingestion (Hours)	Creatinine		ALT (U/L)	INR	Paracetamol (mg/L)	Clinical Scenario
	Initial Ezymatic (µmol/L)	Retrospective LC-MS/MS (µmol/L)				
8	<5	44	17	*	>756	NAC administered, Paracetamol saturating glucuronidation and sulphation metabolism, phase I metabolism induced to form NAPQI
12	<5	37	22	1.1	>756	Paracetamol concentration still above measuring range, INR normal, ALT beginning to rise, Specialist liver unit contacted for advice
21	<5	33	24	1.2	435	Paracetamol concentration dropped, still no useful creatinine concentration, INR rising. Patient transferred to intensive treatment unit (ITU)
25	<5	31	24	1.2	256	Paracetamol rate of metabolism increased, suggest NAPQI production is under control with NAC and haemofiltration
36	22	*	58	1.2	*	Creatinine measurable, low creatinine due to IV fluids and haemofiltration since admision on ITU, increasing ALT and INR
50	20	*	75	1.4	*	INR may increase as a consequence of thrombotic prophylaxis (Heparin) and not hepatic function, ALT increases as expected with stage two toxicity and damage already caused by NAPQI
61	24	*	89	1.2	*	ALT concentration peaked, continued NAC treatment, IV fluids, and haemofiltration
75	20	*	83	1.1	*	
99	25	*	66	1.1	*	6th (final) bag of NAC administered, ALT decreasing and INR at desirable level
120	32	*	60	0.9	*	NAC stopped, INR in desirable range, ALT decreasing, creatinine measurable and rising due to increased mobilisation and removal of IV fluids/ haemofiltration. Patient went on to be discharged under the care of the mental health team with no lasting organ damage.

Table 1., Patient's blood results during admision and treatment of paracetamol overdose. Results highlighted by a green box were generated retrospectively and not released to clinicians during the patient's treatment.

Investigations

To investigate the undetectable creatinine, samples were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS). Comparison (Table 1) confirms the presence of a negative interferent in these samples on ECre.

The samples were freeze, thawed and re-analysed for creatinine (using both ECre and Jaffe creatinine assays). The results of these analyses showed comparable results between ECre and LC-MS/MS (interferent no longer present) and the Jaffe assay had a positive bias compared to LC-MS/MS.

Discussion

There are many factors that may affect creatinine measurement in cases of paracetamol overdose, complicating identification of AKI. The presence of NAPQI in this patient's first four samples could have masked renal injury (Fig 2.).

Treatment with IV fluids and haemofiltration would have aided in treatment of AKI, if present and the efficacy can be seen by the pattern of decreasing creatinine concentrations.

This does raise the question of safety in overdose patients - What can be done to effectively assess renal function?

The use of a Jaffe method may be considered. This does have it's own problems though, the nature of the toxicity leads to build up of multiple interferents for this assay, such as bilirubin, ammonia, and ketone bodies. This can be seen here in Figure 1 where analysis via the Jaffe method was positively biased.

The ideal method of measurement although not practical would be LC-MS/MS. In future practice, there needs to be a greater appreciation for the clinical significance of this interference, how it presents and to inform clinical and laboratory staff to question creatinine results if they do not fit the clinical picture. Suspicion and investigation of interference must be communicated to the clinical team so potential renal injury is not dismissed.

The instability of NAPQI could be utilised to introduce a delayed reanalysis of these samples, but this will require further investigation.

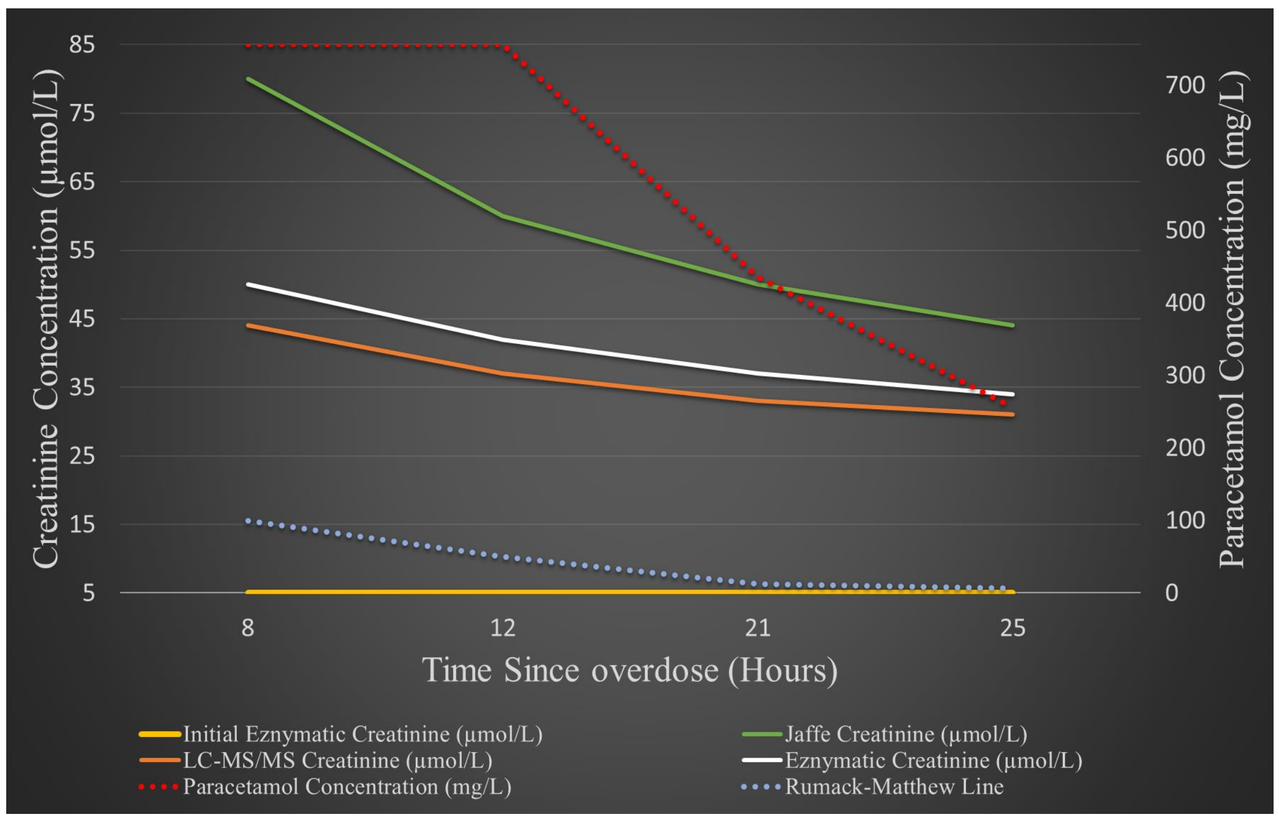

Figure 1., A graph to show the comparison of the retrospective analysis using different creatinine methods and initial creatinine concentrations over the course of paracetamol toxicity

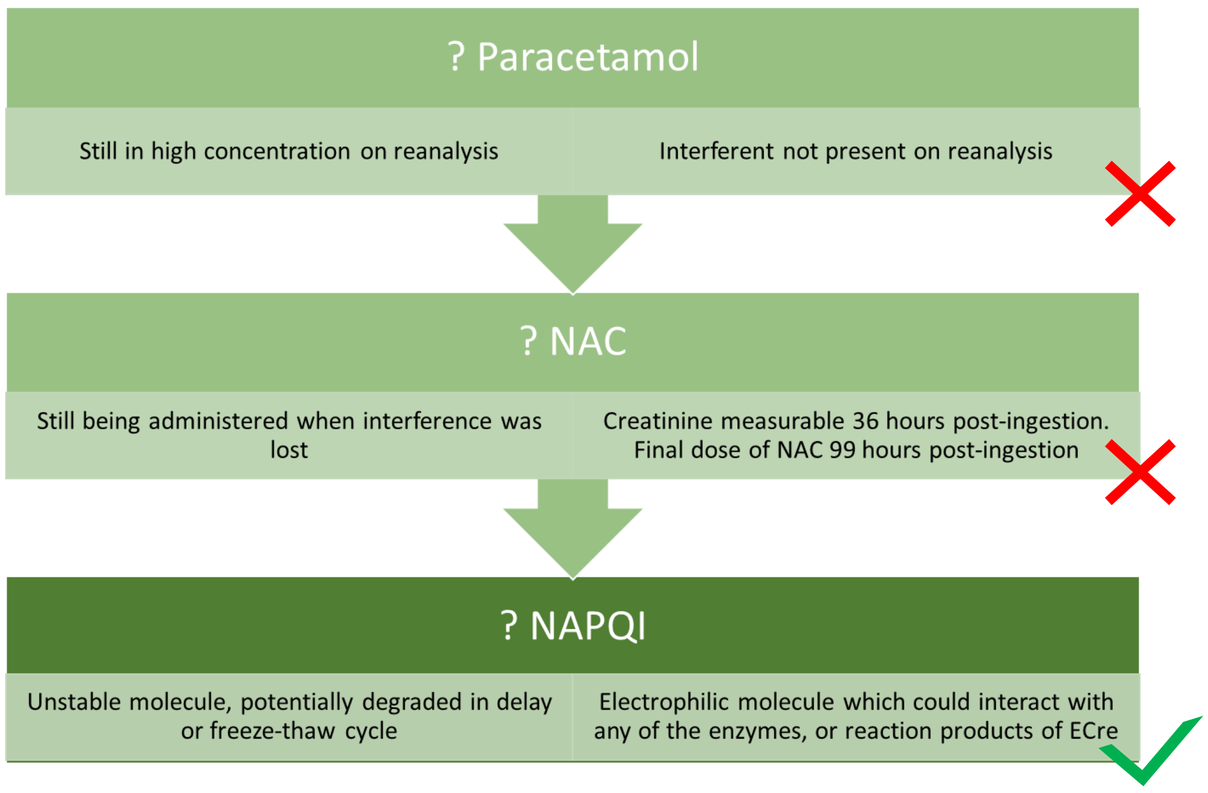

Figure 2., A visual representation of the process of elimination to determine the interferent